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Applicant: Kazuo NAGAI et al.

Title: NOVEL GENE

Appl. No.: 09/786,474

Filing Date: March 5, 2001

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Art Unit: Unassigned

INFORMATION DISCLOSURE STATEMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith on a modified Form PTO-1449 is a listing of documents known to applicant in order to comply with applicant's duty of disclosure pursuant to 37 CFR 1.56. A copy of each listed document is being submitted to comply with the provisions of 37 CFR §§1.97-1.98.

The submission of any document herewith, which is not a statutory bar, is not intended as an admission that such document constitutes prior art against the claims of the present application or that such document is considered material to patentability as defined in 37 CFR §1.56(b). Applicant does not waive any rights to take any action which would be appropriate to antedate or otherwise remove as a competent reference any document which is determined to be a prima facie prior art reference against the claims of the present application.

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TIMING AND RELEVANCE OF THE DISCLOSURE

The instant Information Disclosure Statement if being filed in compliance with 37 CFR §1.97(b) prior to the mailing date of the first official action.

Concise Explanation of Japanese References

Reference A2 [JP-B Sho 62-49038]

A method for producing L-glutamine

Disclosed is a process for producing L-glutamine, in which a microorganism that is a member of the genus Corynebacterium or Brevibacterium and is lysozyme-sensitive and whose L-glutamine production is not suppressed by an excessive amount of biotin present in the medium is cultured in the medium, and in which L-glutamine produced by the microorganism is collected from the culture.

In an attempt to reduce the cost for fermentation by using an inexpensive carbon source such as molass, the inventors searched for mutant strains of Corynebacteria and Brevibacteria that can produce L-glutamine in high yeld in the presence of an excessive amount of biotin. The parental strains Corynebacterium glutamicum ATCC13032 and Brevibacterium flavum ATCC14067 were mutagenized by nitrosoguanidine and subjected to the selection for lisozyme-sensitivity and then for productivity in the presence of excess biotin. As a result, two mutant strains designated Corynebacterium glutamicum KY9703 (FERN-P 4412, NRRL 11271) and Brevibacterium flavum KY9733 (FERN-P 4414, NRRL 11273) were obtained. They can produce glutamine when cultured under conventional conditions (aerobically at 24-37°C, preferably at 28-33°C, at pH 6-9, for 1-3 days). The bacteria showed growth inhibition by lysozyme at the concentrations of 100 µg/ml and 50 µg/ml for Corynebacterium glutamicum KY9703 and Brevibacterium flavum KY9733, respectively, while both the parental strains were inhibited at 800 µg/ml. The L-glutamine accumulations in Corynebacterium glutamicum KY9703 and Brevibacterium flavum KY9733 were 19.0 and 15.0 mg/ml, respectively, in the medium containing 100 µg/ml biotin, while the accumulation was less than 0.1 mg/ml in both parental strain at the same biotin concentration.

Reference 3 [JP-A Sho 54-122794]

A method for producing L-glutamic acid

Disclosed is a method for accumulating L-glutamic acid in the medium by culturing a microorganism that belongs to the genus Corynebacterium or Brevibacterium, in which the microorganism is lysozyme-sensitive and whose L-glutamic acid production is not suppressed by an excessive amount of biotin present in the medium. A lysozyme-sensitive microorganism that belongs to Corynebacterium or Brevibacterium, specifically, Corynebacterium glutamicum KY9703 and KY 9705 (FERN4413, NRRL11272), and Brevibacterium flavum KY9733 are also disclosed. These mutant strains were obtained by mutagenesis using nitrosoguanidine. Growth inhibitory concentrations of these strains were 100, 25 and 50 μg/ml, respectively. They can produce L-glutamate when cultured under conventional conditions (aerobically at 24-37 C°, preferably at 28-33 C°, at pH 6-9, for 1-3 days).

Reference 4 [JP-A Sho 62-44171]

A novel microorganism that produces L-glutamic acid

Two mutant strains of Corynebacterium glutamicum KY9703 and KY 9705, and Brevibacterium flavum KY9733, all of which are lysozyme-sensitive and have ability to produce L-glutamic acid in the presence of excess biotin in the medium, are disclosed and claimed. Descriptions in the specification are almost the same as those in JP-A Sho 54-122794.

Reference 5 [JP-B Hei 01-29555]

A method for producing L-glutamic acid

Disclosed are microorganisms that belong to the genus Corynebacterium or Brevibacterium and is lysozyme-sensitive and whose L-glutamate production is not suppressed by an excessive amount of biotin present in the mediu. They were Corynebacterium glutamicum KY9703, KY9705, T339 and T327, which was obtained by mitagenizing the parental strain Corynebacterium glutamicum ATCC13032, Corynebacterium lilium T322 obtained from the parental strain Corynebacterium lilium ATCC15990, Brevibacterium flavum KY9733 derived from Brevibacterium flavum ATCC14067, and Brevibacterium saccharolticum T321 derived from Brevibacterium saccharolticum ATCC14066. Descriptions in the specification are almost the same as those in JP-A Sho 54-122794.

Reference 6 [Nippon Nogeikagaku Kaishi, Vol. 72 (Abstracts) p. 47 (2A8p12), 1998]

Analysis of Lysozyme-Sensitive Mutants of Coryneform Bacteria

Takasi Hirasawa, Masa-aki Wachi, Kazuo Nagai

Objective: Coryneform bacteria that are used for production of amino acids, such as, inter alia, glutamate, are extremely resistant to detergents and lysozyme. Also, it is known that glutamate production by these bacteria requires treatment by which cell surfaces are affected, such as biotin restriction and penicillin treatment. Thus, in an attempt to solve the cell surface structure of coryneform bacteria, physiological and genetic analysis of a lysozyme-sensitive mutant of Corynebacterium glutamicum was conducted.

Methods and Results: C. glutamicum KY9714, which was isolated as a lysozyme-sensitive mutant, exhibited temperature-sensitive growth at 37°C. The temperature sensitivity suppressed when 20% sucrose was added to the medium. Further, while the wild-type strain showed resistance to SDS, strain KY9714 cultured under the restricted temperature and the penicillin-treated wild-type strain were lysed when SDS was added. These suggest that KY9714 has some defect in the cell wall. Shotgun cloning indexed by complementation of temperature sensitivity allowed us to obtain an EcoRI fragment about 4 kb in length. This fragment complemented the lysozyme-sensitivity as well. These results indicate that lysozyme-and temperature-sensitivities resulted from a single gene mutation.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741.

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Respectfully submitted,

Date July 18, 2001

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